## REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

In gene therapy and artificial substance production systems using living organisms, introducing a nucleic acid, a protein, or such, into the interior of a cell is extremely important. On the other hand, techniques for extracting structures, such as the nucleus of a cell, are also gaining wide attention. In other words, injecting and extracting substances in and out of cells is a fundamental technique of bioengineering.

Conventional substance introduction techniques can be roughly categorized as follows:

(a) Introduction techniques targeting non-specific cell groups; and
(b) Introduction techniques targeting a specific cell.

Examples of technique (a) are those using viral vectors (retroviral vectors, etc.), non-viral vectors (lipofectin, etc.), electroporation, calcium phosphate method, particle-gun method, etc. Technique (b) is exemplified by the microinjection method.

Generally, technique (b) is used against large cells, such as egg cells. One reason for this is that the microinjection method uses the shear force of a capillary glass tube to disrupt the cell membrane, and, therefore, this technique is limited by cell size. Also, this method requires skilled handling, and, therefore, automation is difficult. Furthermore, in many cases, the pipette cannot be inserted due to the flexibility of the cell membrane of normal cells other than egg cells.

In technique (a), a non-specific cell group is randomly treated with the expectation that the added substance will be introduced to a part of the cell group; however, it is very rare that the added substance is introduced into all cells. Further, it is generally difficult to separate cells into which the added substance has been introduced from those cells where no additions were made. Moreover, a sophisticated micromanipulator is required to extract structures within the cell without damaging them and not having drawbacks similar to the above microinjection method.

Thus, at a time when cell treatment has become a routine technique in medicine/engineering, its reproducibility/precision has become an issue. For example, when treating reproductive cells, considering that only an extremely small amount of egg cells can

be obtained from each individual animal compared to somatic cells, an egg cell is a valuable genetic resource. Therefore, the rate of successfully treating them largely depends on the technical experience of the technician.

All prior treatments were limited to single cells, migratory cells, or cells such as cancer cells that could be isolated/reintroduced from/into the body. Therefore, it was extremely difficult to modify treatments so that they could be used for cells that are inseparable from the body, such as nerve cells.

Other than the above examples, the possibility of technological developments resulting from cell modifications is on the increase. A few examples are:

1) To produce cloned animals, it is necessary to inject the nucleus or chromosomal genes via the egg cell membrane, but the success rate of this procedure is extremely low;

2) If a specific cell into which a magnetic structure has been incorporated can be made, it will be possible to magnetically control the location of the cell. The method of introducing magnetic bacteria-derived magnetosome-formation gene is generally used, and, although there are successful examples, in most cases, it is necessary to insert artificial magnetic structures;

3) When preparing micromachines such as microsurgical instruments, it is easily postulated that a cut sufficient to dissect the cell membrane would not be obtained by physical means. Also, membrane disruption using a chemical reaction would be difficult to regulate;

4) In measuring action potential/electric stimulation of nerve cells, the measurement/stimulation can be both carried out extracellularly with electrodes. This weakens the detection signal and increased stimulation input compared to the potential threshold value involved in original neuro activities. If it is possible to implant an electrode within nerve cells, it would make the measurement/stimulation equivalent to the original potential threshold value of nerves possible. For example, in practicing the present invention, kenzan (needlepoint flower holder)-shaped highly accumulated electrode (integral electrode) can be used as a support. When nerve cells are contacted with a membrane-disrupting reagent which is attached to the integral electrode while applying the stimulus to the membranes, the membranes of nerve cells may be perforated and the cellular level connection between electrodes and nerve cells can be achieved. This technique will further enable information exchange between the electrode and nerve with a one-to-one precision.

> 5) In the field of energy conversion engineering, studies on placing micro-photoelectric converting elements onto artificial membranes are being conducted. This approach aims to use the electromotive force given by photoelectric converting elements to generate transmembrane potential. If it becomes possible to place such micro-photoelectric converting elements within cell membranes/mitochondrial membranes, light can be used to supply the energy needed for cellular metabolisms. As a result, it may be possible to use photoenergy just as the plants do.

A fundamental problem underlying these cell treatments is the lack of techniques that would control the disruption of the cell membrane. Various toxins have been examined for use in disrupting the cell; however, those toxins would not respond to the demands of cytoengineering which partially and temporarily disrupts the cell membrane without causing cell death.

In addition, although there are various membrane disruption techniques, those that could site-specifically disrupt a membrane are limited. Microinjectors and micromanipulators cause partial membrane disruption; however, they rely on physical shear force to disrupt and perforate the membrane. Thus, a technique for perforating a membrane without physical shear force while regulating the disruption of the membrane has not been resolved.

The present invention is directed to overcoming the above-noted deficiencies in the prior art.

The rejection of claims 1-5 under 35 U.S.C. § 112 (1st para.), for lack of enablement is respectfully traversed in view of the above amendments and the Declaration Under 37 C.F.R. § 1.132 by Katsuyoshi Ishii ("Ishii Declaration") submitted herewith. Applicants have amended the claims in the present application to recite contacting a membrane with a membrane-disrupting reagent that induces a membrane-denaturing reaction when the membrane is exposed to a stimulus. In addition, as demonstrated in the Ishii Declaration, operable combinations of a "membrane-disrupting reagent" and a "stimulus" would be fully recognized through routine experimentation by one of ordinary skill in the art (Ishii Declaration ¶ 6). For example, it is well known that the initial efficacy of a specific combination of a "membrane-disrupting reagent" and a "stimulus" can be determined by using an injection marker, such as a water-soluble fluorescent dye (Ishii Declaration ¶ 7; page 25, lines 20-31 of the present application). If fluorescence is observed within the cell after treating the cell with the specific combination, that combination is identified as the effective

combination of a "membrane-disrupting reagent" and a "stimulus". It is also well known that the long-term efficacy of a specific combination of a "membrane-disrupting reagent" and a "stimulus" can be determined by using a conventional cell-detachment assay (Ishii Declaration ¶ 8). If the detachment ratio – the ratio of the number of cells that detach from a culture dish after treating the cell with the specific combination to the number of initially cultured cells that remain on the culture dish immediately after treatment – is less than the detachment ratio of a control experiment, that combination is identified as the effective combination of a "membrane-disrupting reagent" and a "stimulus". For all these reasons, the rejection of claims 1-5 for lack of enablement should be withdrawn.

The rejection of claims 1-5 under 35 U.S.C. § 112 (1st para.), for lack of written description of the claimed invention, is respectfully traversed. The U.S. Patent and Trademark Office ("PTO") stated in the outstanding office action that the method of claims 1-5 is not supported in the specification, because the disclosure only teaches one combination of photosensitizing compounds plus light stimulation, while the claims are drawn to method of using combinations of a specific compound plus a specific stimulation. The "membrane-disrupting reagent" of the amended claims is defined as a reagent that induces a membrane-denaturing reaction when the membrane is exposed to a stimulus, and includes, as described at page 9, lines 16-27, enzymes, antibody molecules, membrane bound proteins, glycoproteins, lipids, photosensitizers, oxidants, reductants, explosive compounds, magnetic particulates, metal particles, etc. In addition, the "stimulus" of the amended claims is restricted to those that induce the membrane-denaturing reaction of the membrane-disrupting agent selected and includes electromagnetic waves, particle rays, heat, cooling, electricity, magnetism, oscillations, physical contact,, chemical substances, cells, and viruses, as described at page 9, lines 6-15. Since the elements recited in claim 1 are fully described by the present application, the rejection of claims 1-5 under 35 U.S.C. § 112 (1st para.) should be withdrawn.

The rejection of claims 1-5 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments. Support for the amended claims is found at page 5, lines 2-26, page 6, lines 28-32, page 9, line 28, and page 10, lines 6-22 of the specification.

The rejection of claims 1-6 under 35 U.S.C. § 102(b) as anticipated by Thorpe et al., "Dynamics of Photoinduced Cell Plasma Membrane Injury," Biophysical Journal, 68:2198-2206 (1995) ("Thorpe") is respectfully traversed.

Thorpe discloses a technique to examine in real time the dynamics of membrane rupture in single cells elicited by photodamage. Thorpe teaches that photodamage caused a sudden rupture of the plasma membrane which occurred after a time lag. Both irradiation power density and photosensitizer concentration varied inversely with the time lag. The claims of the present invention, as amended, are directed to "a method of site specific regulated membrane disruption comprising: contacting a membrane with a membrane-disrupting reagent . . . and applying the stimulus to the membrane at a contact site under conditions effective to temporarily and partially disrupt the membrane only at the contact site where permeability of the membrane recovers to the state prior to disruption." Since there is no disclosure in Thorpe of a technique that temporarily and partially disrupts the membrane whereby permeability of the membrane recovers to the state prior to disruption, as claimed, the rejection based on this reference is improper and should be withdrawn.

The rejection of claims 1-6 under 35 U.S.C. § 102(b) as anticipated by Valenzeno, "Photomodification of Biological Membranes with Emphasis on Singlet Oxygen Mechanisms," Photochemistry and Photobiology, 46(1):147-169 (1987) ("Valenzeno") is respectfully traversed.

Valenzeno describes photomodification of biological membranes as well as the role of preillumination association of sensitizers with membranes. Valenzo teaches that if conditions are arranged properly, membranes can be photomodified by sensitizer in the medium external to the membrane, where excited intermediates generated by the sensitizer can diffuse a finite distance in solution, leading to membrane lysis. However, Valenzeno does not disclose or suggest a method where a membrane is temporarily and partially disrupted but where permeability of the membrane recovers to the state prior to disruption, as required by the claims of the present invention. Thus, the rejection based on Valenzeno is improper and should be withdrawn.

The rejection of claims 1-4 and 6 under 35 U.S.C. § 102(b) as anticipated by UK Patent Application No. GB 2 209 468 A to Morgan ("Morgan") is respectfully traversed.

Morgan teaches a method of irradiating liposomes with an incorporated photosensitizing agent with light of the appropriate wavelength so as to effect destabilization of the lipid bilayer and fusion between liposomes and/or exchange of membrane bound constituents of the liposomes between liposomes and/or cells or tissues of a recipient of the liposomes and/or fusion of intact liposomes with such cells or tissues. Morgan is distinguishable from the present invention in that the method for introducing substances by membrane fusion technique as described in Morgan works only in liposomes, where the liposomes are made of the same material as the membrane to be disrupted. In contrast, the present invention relates to a membrane perforation technique, where the membrane to be disrupted is contacted with a membrane-disrupting reagent attached to a support and the support could be made of any material as long as it facilitates precise contact of the membrane-disrupting reagent with the membrane. In other words, the material of the support is not limited to the same material as the membrane to be disrupted, as in Morgan, but could be made of anything including artificial material (e.g. microbeads). Therefore, the rejection based on Morgan is improper and should be withdrawn.

The rejection of claims 1-4 under 35 U.S.C. § 102(b) as anticipated by Sambrook et al., "Molecular Cloning: A Laboratory Manual," 2nd Edition, 16.30-16.31; 16.48-16.53, Cold Spring Harbor Laboratory Press (1989) ("Sambrook") is respectfully traversed.

Sambrook discloses a method of introducing DNA into mammalian cells by fusing protoplasts, prepared from bacteria carrying the plasmid DNA of interest, with cultured cells. Cell fusion is promoted by treating a mixture of the protoplast and mammalian cells with polyethylene glycol (PEG). However, it is well known that PEG nonspecifically denatures membranes. In other words, membrane denaturation reaction caused by PEG is not controlled. Since Sambrook does not disclose or suggest a method of site specific, regulated cell membrane disruption, the rejection based on this reference is improper and should be withdrawn.

The rejection of claims 1-5 under 35 U.S.C. § 102(a) as anticipated by Saito et al., "Light Dose and Time Dependency of Photodynamic Cell Membrane Damage," Photochemistry and Photobiology, 68(5):745-748 (1998) ("Saito") is respectfully traversed in view of the Declaration of Takashi Saitoh ("Saitoh Declaration") and the Declaration of Isao Karube ("Karube Declaration") submitted herewith. As demonstrated in the Saitoh

Declaration and the Karube Declaration, Saito is not prior art under 35 U.S.C. § 102(a). The present invention was conceived and reduced to practice solely by Takashi Saitoh and Isao Karube (Saitoh Declaration ¶ 2; Karube Declaration ¶ 2). Nick A. Hartell, Hitoshi Muguruma, Shu Hotta, Satoshi Sasaki, and Masao Ito, co-authors of Saito, worked under the direction of the named co-inventors and did not contribute to the conception or reduction to practice of the present invention; they were included as co-authors of Saito, because they provided technical assistance (Saitoh Declaration ¶ 2; Karube Declaration ¶ 2). In view of the Saitoh Declaration and the Karube Declaration, it is clear that Saito is not the work of "another", under 35 U.S.C. § 102(a). See In re Katz, 687 F.2d 450, 215 USPQ 14 (C.C.P.A. 1982). As a result, Saito cannot be Section 102(a) prior art with respect to the claimed invention, and the rejection based on this reference should be withdrawn.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: January 11, 2002

Michael L. Goldman
Registration No. 30,727

NIXON PEABODY LLP
Clinton Square, P.O. Box 31051
Rochester, New York 14603
Telephone: (716) 263-1304
Facsimile: (716) 263-1600

| Certificate of Mailing - 37 CFR 1.8(a) | |
|---|---|
| I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on the date below. | |
| Jan. 11, 2002 Date | Ruth R. Smith Ruth R. Smith |

## Appendix A

## Version With Markings to Show Changes Made

In reference to the amendments made herein to claims 1 and 6, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In The Claims:

1. (Amended) A method of site specific regulated membrane disruption [denaturing or perforating a specific site of a membrane, the method] comprising:

contacting [the whole or part of the] a membrane with a membrane-disrupting reagent [containing a specific compound] that induces a membrane-denaturing reaction [by a specific stimulation, and giving said stimulation] when the membrane is exposed to a stimulus, wherein the membrane-disrupting reagent is attached to a support which facilitates precise contact of the membrane-disrupting reagent with the cell membrane; and

applying the stimulus to the membrane at a contact site under conditions effective to temporarily and partially disrupt the membrane only at the contact site where permeability of the membrane recovers to the state prior to disruption.

6. (Twice Amended) [A] The method of claim 1, wherein the [specific stimulation] stimulus is light, and the [compound] membrane-disrupting reagent is a photosensitizing compound.